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COMPARATIVE SUSCEPTIBILITY OF INTRODUCED FOREST-DWELLING MOSQUITOES IN HAWAI'I TO AVIAN MALARIA, *PLASMODIUM RELICTUM*

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ABSTRACT: To identify potential vectors of avian malaria in Hawaiian native forests, the innate susceptibility of *Aedes albopictus*, *Wyeomyia mitchellii*, and *Culex quinquefasciatus* from 3 geographical sites along an altitudinal gradient was evaluated using local isolates of *Plasmodium relictum*. Mosquitoes were dissected 5–8 and 9–13 days postinfective blood meal and microscopically examined for oocysts and salivary-gland sporozoites. Sporogony was completed in all 3 species, but prevalence between species varied significantly. Oocysts were detected in 1–2% and sporozoites in 1–7% of *Aedes albopictus* that fed on infected ducklings. *Wyeomyia mitchellii* was slightly more susceptible, with 7–19% and 7% infected with oocysts and sporozoites, respectively. In both species, the median oocyst number was 5 or below. This is only the second *Wyeomyia* species reported to support development of a malarial parasite. Conversely, *Culex quinquefasciatus* from all 3 sites proved very susceptible. Prevalence of oocysts and sporozoites consistently exceeded 70%, regardless of gametocytemia or origin of the *P. relictum* isolate. In trials for which a maximum 200 oocysts were recorded, the median number of oocysts ranged from 144 to 200. It was concluded that *Culex quinquefasciatus* is the primary vector of avian malaria in Hawai'i.

The endemic avifauna of Hawai'i has suffered great losses since European expansion into the Pacific. More than half of the historically known species have become extinct over the past 200 yr and another half of the remaining species are endangered. Of special concern are the Hawaiian honeycreepers (Drepanidinae), a morphologically and ecologically diverse assemblage of some 50 species that has been reduced to 25 extant species (Freed et al., 1987; James and Olsen, 1991; Jacobi and Atkinson, 1995). Although the causes of these extinctions are many (Banko and Banko, 1976; Atkinson, 1977; Mountainspring and Scott, 1985; Scott et al., 1986; Berger, 1988), introduced mosquito-borne avian disease is now recognized as a key mortality factor in native Hawaiian forest bird populations (Warner, 1968; van Riper et al., 1986; Atkinson et al., 1995, 2000; Feldman et al., 1995).

Mosquitoes were absent from the Hawaiian Islands until their accidental introduction early in the 1800's. The southern house mosquito, *Culex quinquefasciatus*, is thought to have arrived as early as 1826 and was shortly followed by *Aedes aegypti* and *Aedes albopictus* (Van Dine, 1904; Hardy, 1960). Since 1962, 2 additional blood-feeding mosquitoes, *Aedes vexans nocturnus* and *Wyeomyia mitchellii*, have become established in the Hawaiian Islands (Joyce and Nakagawa, 1963; Shroyer, 1981). Only *Cx. quinquefasciatus* and *A. albopictus* are currently found in forest-bird habitat (Goff and van Riper, 1980), but *Wyeomyia mitchellii* could potentially expand its distribution into native forests (Laird, 1988).

Although *C. quinquefasciatus* is presumed to be the sole vector of avian malaria in the Hawaiian Islands, this assumption has been based primarily on the ecology and behavior of Hawai'i's limited mosquito fauna (Warner, 1968; van Riper et al., 1986). All 3 genera, and 4 of the 5 biting mosquito species in Hawai'i, are known to support sporogony of at least 1 species of avian *Plasmodium* (Huff, 1965; Nayar et al., 1980), yet the intrinsic vector competence of Hawaiian mosquitoes to coindigenous isolates of *Plasmodium relictum* has largely gone untested (van Riper et al., 1986).

In the present study, the susceptibility of 3 Hawaiian forest-dwelling mosquitoes to 2 isolates of coindigenous *P. relictum* was compared to determine the relative potential of these species to serve as vectors. A similar comparison was made among *C. quinquefasciatus* collected from 3 distinct geographical sites along an altitudinal gradient to determine if susceptibility was variable within this known vector.

MATERIALS AND METHODS

Plasmodium isolates and inoculum

Plasmodium relictum is the only *Plasmodium* present in the Hawaiian Islands (Laird and van Riper, 1981). Two indigenous isolates of *P. relictum* from native birds were used throughout these experiments. Isolate KV 115 was derived from an infected apapane (*Himatione sanguinea*) captured at Kilauea Crater on the island of Hawai'i in June 1992 (Atkinson et al., 1995). Isolate K1 was derived from pooled blood samples collected from 2 infected elepaio (*Chamsiempis sandwichensis*) captured along the Alakai Swamp Trail on the island of Kauai in September 1995 (Atkinson et al., 2001). Both isolates were initially passaged into an uninfected canary (*Serinus canaria*). At peak parasitemia, infected blood was then collected, cryoprotected with glycerin, and stored in liquid nitrogen. Frozen aliquots were later thawed, degylcerinated, and passaged intravenously (i.v.) into Pekin ducklings (*Anas platyrhynchos*). Subsequent serial i.v. passages used in feeding trials were K1 passages 11, 16, and 17, and KV 115 passage 34. Initial experiments were conducted with the Kauai (K) isolate of *P. relictum* until an Island of Hawaii isolate (KV) became available.

Mosquitoes

Colonies of *Cx. quinquefasciatus* were started from egg rafts and larvae collected from 3 sites on the east flank of Mauna Loa, Island of Hawai'i. Collections were made from flower vases in a coastal cemetery at Puna (19°25′10.2″N, 154°53′16.3″W, elevation 12 m), from oviposition basins set out at the Pana'ewa Zoo (19°38′51.1″N, 115°04′10.5″W, elevation 150 m), and from refuse tires at Keauhou Ranch (19°26′45.8″N, 155°17′02.1″W, elevation 1,250 m). *Culex quinquefasciatus* used in experiments were from field-collected eggs and larvae or from the F3–F10 generations of established colonies. Colonies were maintained at 28 C, 70–80% RH, and 12 : 12 light : dark cycle. Larvae were reared at uniform densities and fed ad libatum a 1 : 1 mixture of powdered yeast extract and lactalbumin (ICN Biomedicals, Inc., Aurora, Ohio). Adult *Cx. quinquefasciatus* were maintained on a 10% sucrose solution and blood fed on chicken blood through an artificial membrane (Harada et al., 1996). *Aedes albopictus* were collected in the field as biting adults from 2 locations, Lyon Arboretum, O'ahu (21°20′10.1″N, 157°48′16.8″W) and Onomea Bay, Island of Hawai'i (19°48′28.9″N, 155°05′31.5″W). *Wyeomyia mitchellii* were collected in the field as bit-

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TABLE I. Various parameters of the susceptibility feeding trials.

Trial no.	Experimental source of mosquito species/population (altitude)					*Plasmodium relictum*		
	Culex quinquefasciatus							
	Puna (12 m)	Pana'ewa (150 m)	Keauhou (1250 m)	*Aedes albopictus*	*Wyeomyia mitchellii*	Isolate (passage no.)	Duck ID no.	Percent gametocytemia
1	Field eggs	Field eggs	Colony F1	Colony F1*	Field larvae*	K1 (P11)	93	4.9
							96	3.7
							98	6.8
							99	4.7
2	None	None	Colony F3	Colony F1*	Field larvae*	K1 (P17)	30	0.04
							31	0.05
							32	0.09
3	Field eggs	Colony F7	Colony F7	None	None	K1 (P16)	87	1.0
							88	1.4
							100	0.5
4	Field eggs	Field eggs	Colony F10	Colony F1†	Field adults*	KV (P33)	74	3.7
							75	2.0

* *Wyeomyia mitchellii* and *A. albopictus* were collected as biting adults or larvae in Lyon Arboretum, O'ahu (altitude 220 m).
† *Aedes albopictus* collected as biting adults in Onomea, Island of Hawai'i (altitude 40 m).

ing adults or as larvae inhabiting tank bromeliads in Lyon Arboretum, O'ahu.

Experimental mosquitoes were reared under colony conditions, except adults were maintained on a 3% sucrose solution and were blood fed on an infected host 7–14 days after emergence. Mosquitoes were deprived of sucrose for 24 hr and water for 12 hr prior to blood feeding on infected ducklings. Due to a prolonged and asynchronous larval development, it was difficult to rear large numbers of adult *W. mitchellii* for individual feeding trials. For this reason, field-captured adult *W. mitchellii* were used directly in the feeding trial 4. Fifteen, field-captured adults were also dissected to determine field parity and infection status.

Experimental hosts and mosquito blood feeding

Four- to 7-day-old Pekin ducklings (*A. platyrhynchos*) (Metzger Farms, Petaluma, California) were maintained on chicken starter and housed in a commercial brooder or battery cage equipped with a heat lamp. For each feeding trial, infection was established in 3–4 ducklings by i.v. passage of freshly collected, heparinized whole blood from a single infected duckling. Every afternoon, starting 2 days after inoculation, ducklings were bled from a leg vein and a thin blood smear was made. Blood smears were fixed with methanol, stained with phosphate buffered Giemsa, and examined by light microscopy at ×1,000. Percent total parasitemia and gametocytemia were based on a total count of 10,000 cells per slide (Godfrey et al., 1987). At peak parasitemia, ducklings were shaved on the breast and anesthetized with an intramuscular (i.m.) injection of Telazol® (13 mg/kg; Carp et al., 1991). Ducklings seldom remained immobilized for more than 30 min but could be safely redosed. Anesthetized ducklings were placed on foam pads within screened mosquito cages (30 × 30 × 30 cm) (BioQuip, Gardena, California), where mosquitoes fed on the feet, bill, eyelid, and shaved breast. In feeding trials with older, larger ducklings, mosquitoes were confined in mesh-topped 0.5-L containers that were placed against the shaved breast of the anesthetized host. Blood feeding commenced at 1600 hr to accommodate diurnal feeders and ended at 2400 hr.

Feeding trials and mosquito dissections

Four separate feeding trials were conducted (Table I). For each feeding trial, approximately half of the mosquitoes were dissected 5–8 days after the infective bloodmeal. Remaining mosquitoes were dissected 4–5 days later. The estimations for development time were based on previous studies with *P. relictum* and *Cx. quinquefasciatus* (LaPointe 2000) and from studies using similar mosquito genera with different *Plasmodium* species (Nayar et al., 1980; Thathy et al., 1994). Based on the available literature, it was assumed that at least oocysts would be detectable 9–13 days after the infective blood meal. Mosquitoes were dissected in 0.9% saline and midguts were stained with 1% mercurochrome (Eyles, 1950) to enhance detection of oocysts. Midguts and salivary glands were transferred in saline to a clean slide, covered with a coverslip, and examined under a light microscope at ×400 for evidence of infection. The total number of oocysts per midgut (oocyst intensity) was recorded except in feeding trial 1; oocyst intensity was so great in most cases that only a maximum of 200 oocysts was counted. The decision to cap counts was based on the limited time available to complete dissections and the assumption that differences in oocyst intensity above 200 oocysts would not have a functional impact on transmission. Salivary glands were gently squashed and sporozoite intensity was scored as the approximate number of sporozoites in 1 positive field with 1 = 1–100, 2 = 101–500, and 3 = 501–1,000. The percent relative sporozoite intensity was the proportion of sporozoite-positive individuals in each of the 3 intensity classes. Mosquito susceptibility was expressed as the prevalence (percent) of oocyst- and sporozoite-infected individuals in experimental groups. Oocyst prevalence and intensity were based on early dissections (5–8 days), whereas sporozoite prevalence and intensity was restricted to later dissections (9–13 days).

Data analysis

Mosquito availability, gametocytemia, and the *P. relictum* isolate varied during the 4 feeding trials (Table I). For interspecific comparisons, only *Cx. quinquefasciatus* from the Keauhou colony were used in the analysis. Because gametocytemias differed by a factor of 100 between trials 1 and 2, each species was analyzed separately for the effect of gametocytemia on prevalence. Feeding trials were pooled for determining overall prevalence when mosquito source and parasite isolate did not vary or when gametocytemia was shown to have no significant effect. Mosquitoes from trial 4 were not pooled with other trials but used to test for differences in susceptibility between *Cx. quinquefasciatus* Keauhou with the Island of Hawaii (KV) and Kauai (K) isolates of *P. relictum*. Statistical comparisons of prevalence between species, host gametocytemias, parasite isolates, and geographical sites along an altitudinal gradient for *Cx. quinquefasciatus*, were made by Pearson's chi-square test and Fisher's exact test.

Oocyst number varied considerably between trials, and frequency distributions were heavily skewed to the right. For this reason, the distribution of oocyst number is describe here as a median and range. For interspecific and intraspecific analysis, only the counts from *Culex* in trial 1 were used. Differences in intensity were evaluated by Kruskal–

TABLE II. Midgut (oocysts) and salivary gland (sporozoites) prevalence and intensity in 3 forest-dwelling mosquitoes dissected after feeding on *Plasmodium relictum*-infected ducklings during Trials 1 and 2.

Species	Prevalence: No. infected/no. dis. (% infected)		Oocyst no.* median (range)	Percent relative sporozoite intensity†		
	Oocysts	Sporozoites		1	2	3
Aedes albopictus	1/97 (1)	1/95 (1)	5	100	0	0
Wyeomyia mitchellii	4/21 (19)	2/27 (7)	2 (2–16)	100	0	0
Culex quinquefasciatus	65/73 (89)	83/97 (86)	190 (4–200)	39	43	18

* Based on infected mosquitoes, where *Aedes* N = 1, *Wyeomyia* N = 4, and *Culex* N = 20. From Trial 1 only, where the maximum number of oocysts per midgut counted was 200.
† The proportion of sporozoite-positive individuals in each of the 3 intensity classes. Based on infected mosquitoes, where *Aedes* N = 1, *Wyeomyia* N = 2, and *Culex* N = 83.

Wallis test and Mann–Whitney *U*-test. All statistical tests were performed with SYSTAT® statistical software (1997).

RESULTS

Sporogony was completed in all 3 species of mosquitoes although the degrees of susceptibility, as evidenced by prevalence, were significantly different (oocysts: $\chi^2 = 142.11$, df = 2, $P < 0.001$; sporozoites: $\chi^2 = 156.86$, df = 2, $P < 0.001$) (Table II). Only 1% of the *A. albopictus* (Island of Hawai'i) had oocysts or sporozoites. Intensity was low in both the single-infected midgut (5 oocysts) and in the single-infected salivary gland (≤100 sporozoites). *Wyeomyia mitchellii* was slightly more susceptible than *A. albopictus*. Nineteen percent of early dissections were positive for oocysts. Between 2 and 16 oocysts were observed per infected midgut. The sporozoite prevalence was only 7%. Sporozoite intensity was low (≤100) and, in 1 mosquito, sporozoites appeared short, thickened, and crescent shaped. *Culex quinquefasciatus* was found to be highly susceptible to *P. relictum*. Oocyst prevalence for mosquitoes from the Keauhou colony of *Cx. quinquefasciatus* was 89% and the median number of oocysts per infected midgut was 190. This latter number represents the results of trial 1 only and is a conservative estimate because the actual number of oocysts was often well in excess of the arbitrary maximum of 200. Sporozoite prevalence was equally high (90%), and more than half of the salivary-gland infections had a sporozoite intensity greater than 100. Sporozoite-infected salivary glands were first observed in dissections of *Cx. quinquefasciatus* on day 6. By day 7, most salivary glands were infected.

Because a different isolate of *P. relictum* and alternate sources of *A. albopictus* and *W. mitchellii* were used in trial 4, it was not pooled with trials 1 and 2. In general, however, similar results were obtained (Table III). Prevalence of oocysts and sporozoites were significantly different between the 3 species (oocyst: $\chi^2 = 113.33$, df = 2, $P = 0.0005$; sporozoites: $\chi^2 = 34.56$, df = 2, $P = 0.0005$). *Aedes albopictus* and *W. mitchellii* in trial 4 had slightly higher prevalences of oocysts and sporozoites than mosquitoes pooled from trials 1 and 2 (Table III). Sporozoite prevalence for Keauhou *Cx. quinquefasciatus* in trial 4 was notably less than those of the pooled trials; however, the considerably smaller sample size for *Cx. quinquefasciatus* may account for this variation. Because wild adult *Wyeomyia* were used in trial 4, a small sample (N = 15) of wild *Wyeomyia* was dissected before the experiment. Although 60% of these mosquitoes were parous, no natural infections were found. The comparison of *Cx. quinquefascitus* Keauhou infected with the KV isolate and those infected with the K isolate indicated no significant differences in oocyst prevalence (Fisher's exact test, $P = 0.268$) or sporozoite prevalence (Fisher's exact test, $P = 0.195$).

No significant differences were observed in the prevalence of oocysts (Fisher's exact test, $P = 0.244$) or sporozoites (Fisher's exact test, $P = 0.774$) in *Culex* that fed on ducks with high (trial 1) and low (trial 2) gametocytemia. *Aedes albopictus* that fed on a low gametocytemic host did not become infected, whereas 3% (oocyst) and 2% (sporozoite) of those feeding on a high gametocytemic host did become infected. These differences were not significant (Fisher's exact test: oocyst, $P =$

TABLE III. Midgut (oocysts) and salivary gland (sporozoites) prevalence and intensity of 3 forest-dwelling mosquitoes dissected after feeding on *Plasmodium relictum*-infected ducklings during trial 4.

Species	Infection rate: No. infected/no. dis. (% infected)		Oocyst no.* median (range)	Percent relative sporozoite intensity†		
	Oocysts	Sporozoites		1	2	3
Aedes albopictus	1/59 (2)	3/42 (7)	5	33	33	33
Wyeomyia mitchellii	3/46 (7)	1/15 (7)	1 (1–41)	100	0	0
Culex quinquefasciatus	32/33 (97)	15/21 (71)	77 (1–1087)	27	0	73

* Based on infected mosquitoes, where *Aedes* N = 1, *Wyeomyia* N = 3, and *Culex* N = 32.
† The proportion of sporozoite-positive individuals in each of the 3 intensity classes. Based on infected mosquitoes, where *Aedes* N = 3, *Wyeomyia* N = 1, and *Culex* N = 15.

TABLE IV. Midgut (oocysts) and salivary gland (sporozoites) prevalence and intensity of 3 altitudinal populations of *Culex quinquefasciatus* dissected after feeding on *Plasmodium relictum*-infected ducklings during trials 1 and 3.

Populations	Infection rate: No. infected/no. dis. (% infected)		Oocyst no.* median (range)	Percent relative sporozoite intensity†		
	Oocysts	Sporozoites		1	2	3
Puna	74/79 (94)	44/52 (85)	144 (1–200)	18	18	64
Pana'ewa	59/60 (98)	70/72 (97)	200 (7–200)	13	40	47
Keauhou	51/55 (93)	82/92 (89)	190 (4–200)	16	32	52

* Based on 20 infected mosquitoes per population. (Trial 1 only). Maximum number of oocysts per midgut counted was 200.
† The proportion of sporozoite-positive individuals in each of the 3 intensity classes. Based on infected mosquitoes, where Puna N = 44, Pana'ewa N = 70, and Keauhou N = 82.

0.412; sporozoite, $P = 0.441$). Twenty-nine percent of *W. mitchellii* (N = 14) feeding on a low-gametocytemic host became infected, whereas no infection was detected in mosquitoes (N = 7) feeding on a high-gametocytemic host. Sample sizes were quite low for this species and the difference was not statistically significant (Fisher's exact test, $P = 0.255$). Prevalence of sporozoites in *W. mitchellii* that fed on hosts with high and low gametocytemia did not differ (Fisher exact test, $P = 1.000$). Not surprisingly, we found that, in *Cx. quinquefasciatus*, the median number of oocysts per infected midgut was related to host gametocytemia. Significantly more oocysts were observed in *Cx. quinquefasciatus* Keauhou that fed on high-gametocytemic hosts (trial 3, N = 10, median = 411.50, range 55–962) than those that fed on low-gametocytemic hosts (trial 2, N = 39, median = 7, range 1–170) (Mann–Whitney *U*-test = 376, $P < 0.001$).

Culex quinquefasciatus from all 3 geographical sites were highly susceptible to *P. relictum* (Table IV). There was no significant difference in the prevalence of oocysts among individuals from each of these sites ($\chi^2 = 2.221$, df = 2, $P = 0.329$). There was, however, a significant difference in the prevalence of sporozoites among these groups ($\chi^2 = 6.206$, df = 2, $P = 0.045$). *Culex quinquefasciatus* from Puna (altitude 12 m) and Keauhou (altitude 1,250 m) had lower observed sporozoite prevalences (85 and 89%, respectively) than those from Pana'ewa (altitude 150 m) (97%). Differences were also found in the intensity of infection (Table IV). *Culex quinquefasciatus* from Puna had a lower median number of oocysts per infected midgut than mosquitoes from Keauhou or Pana'ewa (Kruskal–Wallis test = 8.977, $P = 0.011$).

DISCUSSION

Since Warner's (1968) initial investigation, it has been assumed that *Cx. quinquefasciatus* is the primary, if not sole, vector of avian malaria in the Hawaiian Islands (van Riper et al., 1986). In lieu of field-infected mosquitoes, the basis for this assumption has been largely ecological and supported in part by limited transmission studies and the literature (Warner, 1968; van Riper et al., 1986). *Culex quinquefasciatus* and its temperate analog, *C. pipiens*, have long been considered the classical vectors of *P. relictum*. Numerous laboratory studies have demonstrated the high susceptibility of these species to avian malaria (Huff, 1965), making them important experimental models during the first half century of malaria research (Hewitt, 1940; Harrison, 1978). Reeves and coworkers (1954), however, found significantly more naturally infected *Culex stigmatosoma* and *Culex tarsalis* than infected *Cx. quinquefasciatus* in a study area where the dominant avian malaria was *P. relictum* (Herman et al., 1954). Furthermore, both *Cx. stigmatosoma* and *Cx. tarsalis* proved more susceptible (100 and 98%, respectively) than *Cx. quinquefasciatus* (50%) in laboratory experiments to a local isolate of *P. relictum* (Rosen and Reeves, 1954). At least 25 other mosquitoes, including 5 anophelines, have been found to support the complete sporogony of *P. relictum* in laboratory-susceptibility trials (Huff, 1965; Work et al., 1990), making it possible that 1 or more other introduced mosquitoes may serve as important secondary vectors in Hawai'i.

Aedes albopictus supports the development of and transmits *Plasmodium fallax*, *P. gallinaceum*, and *P. lophurae* in laboratory experiments (Huff, 1965). Attempts to investigate the susceptibility of *A. albopictus* to *P. relictum* in Hawai'i by van Riper and coworkers (1986) failed when this species refrained from feeding on an infected host. In the current study, *A. albopictus* fed readily on infected ducklings. Although sporogony of *P. relictum* was completed in a few individuals, *A. albopictus* appears to be quite refractory. Extremely low oocyst prevalence and intensities, but successful development of sporozoites and infection of salivary glands, suggests that refractory mechanisms are primarily directed at the gametocyte or ookinete stages. Melanotic encapsulation of oocysts is a documented immunological response to *Plasmodium* ssp. invasion (Collins et al., 1986; Paskewitz et al., 1988; Beier, 1998), but no encapsulated oocysts were observed in the 293 dissections of *A. albopictus* in the present study. Vaughan and colleagues (1994) found that the relationship of ookinete density to oocyst density was linear for the highly susceptible *Anopheles freeborni* and curvilinear for the significantly less susceptible *Anopheles gambiae*. They proposed that the peritrophic matrix acts as an impenetrable barrier until a threshold density of ookinetes is achieved to explain this curvilinear relationship. Infections in *A. albopictus* were observed only in trials where the gametocytemia exceeded 1%, suggesting that a similar refractory mechanism may exist for *A. albopictus*.

The results with *W. mitchellii* are perhaps the most intriguing, though probably the least reliable, due to limited sample size. Both oocysts and salivary-gland sporozoites were observed in this species at prevalences and intensities greater than *A. albopictus* but well below those recorded for *Cx. quinquefascia-*

tus. This is only the second time that complete sporogony of a *Plasmodium* sp. has been reported in a Sabethine mosquito (Nayar et al., 1980). The relative difference in oocyst and sporozoite prevalences suggests an inability for sporozoites to locate or penetrate the salivary glands (Beier, 1998). Nayar and coworkers (1980) reported oocyst prevalence of 35–85% for *Wyeomyia vanduzeei* that fed on turkey poults infected with *P. hermani*. They also observed that sporozoite-infected salivary glands were not as prevalent and did not persist over time. Although inoculation of sporozoites did produce infection in turkey poults, attempts to transmit infection by infective mosquito bites failed (Nayar et al., 1980). In the present study, salivary-gland sporozoites in some *W. mitchellii* appeared to have an aberrant morphology that may be an indication of inviability.

From an ecological standpoint, *W. mitchellii* is the least likely vector of avian malaria in Hawai'i. Its current distribution in the islands appears to be restricted by larval habitat (leaf axils) and, as yet, is only found in residential and horticultural areas where ornamental bromeliads and aroids are grown (Shroyer, 1981). There is some concern, however, that this species might expand its range into native forest by utilizing the leaf axils of native aroids, bird-nest ferns (*Asplenium nidus*), and the endemic climbing Pandanales ieie (*Freycinetia arborea*) (Laird, 1988). *Wyeomyia mitchellii* is an avid diurnal biter and, in Florida, this species was reported to feed on rabbits almost exclusively (Edman and Haeger, 1977). Given the distributional and feeding ecology of this mosquito, along with the demonstrated low susceptibility to *P. relictum*, *W. mitchellii* is probably little more than a curiosity in the epizootiology of avian malaria in Hawai'i.

A similar argument can be made for *A. albopictus*, although this species is readily found in lowland (<300 m) forest habitat and may occasionally be found in forests as high as 900 m in elevation (Goff and van Riper, 1980). Tempelis and coworkers (1970) found Hawaiian *A. albopictus* blood fed almost exclusively on mammals. As with *W. mitchellii*, the diurnal feeding habits of *A. albopictus* would be a hindrance to successful blood feeding on a passerine host (Edman et al., 1974). Even in lowland forests where high densities of this mosquito can be found, the highly refractory nature of *A. albopictus*, combined with its host feeding preferences, make this species an incidental vector of avian malaria at best.

The current susceptibility studies and ecological profile, however, strongly support *Cx. quinquefasciatus* as the vector of *P. relictum* in the Hawaiian Islands. Oocyst and sporozoite prevalence observed in the present study are among the highest reported for *Cx. quinquefasciatus*, as well as other species of mosquitoes, exposed to avian malaria (Herman et al., 1954; Huff, 1965; Beier and Trpis, 1981; Christensen et al., 1983; Work et al., 1990). The suitability of *Cx. quinquefasciatus* as a vector was also evident in its ability to acquire infections when host gametocytemia was quite low and to support massive infections of parasites when host gametocytemia was high. It was not uncommon to find single midguts with an excess of 1,000 oocysts in mosquitoes that had fed on a high (>1%) gametocytemic host. Oocyst intensity (oocyst index) is a common parameter measured in comparative studies and is a good indicator of susceptibility since most refractory mechanisms appear to affect gametocytes and ookinetes (Beier, 1998). A more important parameter in determining vector status, however, is the sporozoite intensity. In all trials with *Cx. quinquefasciatus*, only a few oocyst-infected mosquitoes failed to produce salivary-gland infections of at least median sporozoite intensity.

Although some differences in susceptibility were observed among *Cx. quinquefasciatus* collected along an altitudinal gradient, mosquitoes from all 3 sites proved highly susceptible (>80%) and it seems unlikely that parasite transmission would be significantly reduced by this variation. Intraspecific variation in susceptibility has been well documented among laboratory-selected strains (Huff, 1929; Warren et al., 1977; Chan et al., 1994) but seldom examined in natural geographical strains (Collins et al., 1976). The present findings are somewhat similar to those of Collins et al. (1976), where significant differences in susceptibility to *P. vivax* were found among *Anopheles albimanus* collected across relatively short geographical distances in El Salvador. Their results were limited to oocyst susceptibility while our results demonstrate susceptibility changes in oocyst number and sporozoite prevalence. Whereas genetic isolation was not previously suspected, there is recent molecular evidence to suggest limited gene flow between populations of *Cx. quinquefasciatus* on the Island of Hawai'i (Fonseca et al., 2000). Although we cannot rule out direct selection for refractoriness, the observed differences in innate susceptibility in the current study may best be explained by genetic drift (Yan et al., 1997) or a genetically associated environmental factor, such as insecticide resistance (McCarroll et al., 2000). Our finding of no differences in the mosquito susceptibility between geographically distinct isolates is consistent with conclusions drawn from host cross-resistance experiments using the same isolates (Atkinson et al., 2001). Results from both studies suggest these isolates are biologically similar and may represent a homogenous Hawaiian Island strain.

The ecological attributes of *Cx. quinquefasciatus* in Hawai'i compliment the observed high susceptibility and are consistent with a vector of avian disease. *Culex quinquefasciatus* has a natural host association with passerine birds that has been demonstrated through blood-meal analysis in both the southern continental United States (Edman, 1974; Niebylski and Meek, 1992) and Hawai'i (Tempelis et al., 1970). It is also known to occur year round in forest-bird habitat from sea level to 1,500 m (Goff and van Riper, 1980; LaPointe, 2000). However, the most critical field evidence to support *Cx. quinquefasciatus* as the primary vector comes from the dissection of wild-captured mosquitoes. LaPointe (2000) found natural oocyst prevalence ranging from 3 to 41% in *Cx. quinquefasciatus* collected from forest-bird habitat.

In light of the present susceptibility data and ecological profiles for the 3 forest-dwelling mosquitoes occurring in Hawai'i, it is concluded that *Cx. quinquefasciatus* is the primary vector of *P. relictum* and that concerns over important secondary or yet unknown vectors are largely unfounded. Future strategies to reduce the transmission of avian disease through vector control should focus on this species.

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